UNIVERSITI PUTRA MALAYSIA

CONCENTRATION OF SERUM AMYLOID A IN CLINICALLY NORMAL ENDURANCE HORSES IN MALAYSIA

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CONCENTRATION OF SERUM AMYLOID A IN CLINICALLY NORMAL ENDURANCE HORSES IN MALAYSIA

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A project paper submitted to the

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Concentration of Serum Amyloid A in Clinically Normal Endurance Horses in Malaysia", by Sujey Kumar Rajendren and in our opinion it is satisfactory in term of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4901 – Project.

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DEDICATION

Every challenging work needs self-efforts as well as guidance of elders especially those who were very close to our heart.

My humble effort I dedicate to my loving

FATHER & MOTHER,

Whose affection, love, encouragement and prays of day and night make me able to finish my task,

Along with all hard working and respected

SUPERVISORS

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to both my supervisors, Dr. Nurul Hayah Khairuddin and Dr. Sumita Sugnaseelan, for their excellent guidance, caring, patience and providing me with an excellent atmosphere for doing research. I would like to thank Dr. Reuben Sunil Kumar Sharma for letting me use parasite laboratory for running my ELISA analysis.

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4901 – Projek

KEPEKATAN SERUM AMILOID A DI DALAM KUDA ENDURAN YANG SIHAT DARI SEGI KLINIKAL DI MALAYSIA

Oleh

Sujey Kumar Rajendren

Penyelia: Dr. Nurul Hayah Kharuddin

Penyelia bersama: Dr. Sumita Sugnaseelan

Kuda enduran yang menjalani latihan berat secara berterusan akan mengalami proses inflamasi disebabkan oleh tindakbalas fasa akut dengan pengeluaran protein fasa akut, iaitu serum amiloid A (SAA). Kajian ini dikendalikan untuk mengenalpasti kepekatan SAA dalam kuda enduran yang sihat dari segi klinikal yang direhatkan daripada perlumbaan enduran. Kepekataan SAA ditentukan melalui penilaian sampel *sera* daripada 40 kuda dari 3 kandang, secara *two-site enzyme linked immunoassay* (EIA).

Kepekatan tertinggi SAA diperhatikan dalam kuda yang direhatkan di antara 12 dan 24 bulan, manakala kuda yang direhatkan lebih daripada 24 bulan mempamerkan kepekatan SAA yang terendah. Semua kuda yang berumur di antara 6 dan 11 tahun mempunyai kepekatan SAA tinggi. Apabila selang berehat dibandingkan dengan jantina, ia menunjukkan bahawa semua kuda betina mempunyai kepekatan SAA tinggi berbanding *gelding* dan *stallion*. Kuda yang direhatkan di antara 12 dan 24 bulan boleh dipertimbangkan cenderung untuk mendapat kecederaan kerana mungkin ada pengurangan kecergasan dalam kuda-kuda ini. Kepekatan SAA dalam kuda direhatkan lebih daripada 24 bulan adalah yang paling

mungkin rendah disebabkan oleh kuda telah pulih dengan baik dari kecederaan yang mungkin dialami semasa perlumbaan sebelumnya. Kepekatan SAA kuda *Thoroughbred* adalah lebih tinggi berbanding dengan kuda *Arabian* pada setiap jangka masa rehat

Kepekatan SAA yang disarankan sebagai rujukan untuk kuda enduran di Malaysia ialah antara 2.09 dan 8.09 mg/l. Kepekatan SAA dalam kuda enduran di negara lain di jangka lebih rendah kerana cuaca di Malaysia yang agak panas dan laluan enduran lebih mencabar.

Kata kunci: Serum amiloid A, kuda enduran, latihan berat, umur, jangka masa rehat.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901 - Project

CONCENTRATION OF SERUM AMYLOID A IN CLINICALLY NORMAL ENDURANCE HORSEs IN MALAYSIA

by

Sujey Kumar Rajendren

2015

Supervisor: Dr. Nurul Hayah Khairuddin

Co-supervisor: Dr. Sumita Sugnaseelan

Endurance horses which undergo continuous strenuous training may experience injuries giving rise to inflammation which leads to an acute phase reaction with the production of acute phase protein, namely serum amyloid A (SAA). This study was conducted to establish the concentration of SAA in clinically normal endurance horses while they were rested from endurance racing. The concentration of SAA was ascertained *via* sampled sera from 40 individuals at three stables, using a two-site enzyme linked immunoassay (EIA) test.

The highest concentration of SAA was observed in horses rested between 12 and 24 months, while horses that were rested more than 24 months expressed the lowest concentration of SAA. All horses between the ages of 6 and 11 years old had high SAA concentration. When resting intervals were compared against gender, it showed that all mares had high SAA concentration compared to geldings and stallions. Horses that were rested between 12 and 24

months may be considered prone to injuries as there may be a reduction in fitness in these horses. The SAA concentration in horses rested more than 24 months was low most probably due to the horses having recovered well from possible injuries sustained during an endurance race. SAA concentration of Thoroughbred horses were comparatively higher than Arabian horses at all rest intervals

The recommended SAA reference range for the endurance horses in Malaysia is between 2.09 mg/l and 8.09 mg/l. As the endurance terrain and weather are tough in Malaysia, it is expected that the SAA concentration in endurance horses in Malaysia may be higher than other places.

Keyword: Serum Amyloid A, endurance horse, strenuous training, age, rest interval

1.0 INTRODUCTION

Endurance riding is a competition to test the rider's ability to safely manage the stamina and fitness of his horse over an endurance course in a race against the track, distance, climate, terrain and time. It was first developed in the early 1900s as a military test for cavalry mounts, where horses were required to go up to 300 miles ride while carrying 200lbs for five days. The oldest organised long distance endurance competition was probably the Vienna-Berlin ride in 1892 (Nagy *et al*., 2012). The cavalry test became a civilian sport in the early 1950s.

The first modern endurance ride held in 1955 was a one day, 100 miles race from Lake Tahoe, California to Auburn California. On the original course, the fastest winning speed was 15 km/hour (Nagy *et al*., 2012). Australia holds the second oldest tradition of modern endurance competition, having run the 160 km Tom Quilty Gold Cup since 1966. Spain and Portugal have held endurance rides since the 1950s.

The European Long Distance Rides Conference (ELDRIC) was formed in 1979 and it played an important role in integrating North American, Australian and South African guidelines, and organising international competitions. Endurance became a Fédération Equestre Internationale (FEI) discipline in 1982, and since then international rides have been conducted under FEI regulations (Nagy *et al*., 2012). The basic rule for endurance riding is the first horse to finish in acceptable condition, is the winner and there is no minimum amount of time allowed for the race, giving horse and rider an opportunity to set a quick pace. The horses are supervised by veterinarians at each vet gate.

Endurance horses have a physical look which could be described as small and without excessive muscling. Typical body weight measurements would be in the range of 850 to 1050 pounds. This type of body is advantageous both for reducing thermal load and for travelling up and down mountains (Duren & Duren, 1955).

An endurance horse ridden at a medium trot (250 meter/min) could potentially complete a 25-mile endurance course in about 3 hours, a 50-mile endurance course in just over 5 hours and a 100-mile course in approximately 11 hours (Duren & Duren, 1955). Given these estimates for "competition time," a tremendous chance exists for occurrence of inflammation on the musculoskeletal system. Monitoring the inflammatory response is a clinical challenge, because the classical galenic clinical signs of *dolor, rubor, calor, tumor* and *functio laesea* are not always clinically manifested (Jacobsen *et al*., 2007). Recognising inflammation in a horse with a purulent wound usually requires nothing more than a clinical assessment, but in horses that do not display obvious clinical signs yet remain ill, blood-biochemical and/or haematological testing may be necessary in order to identify inflammatory disease.

The search for blood parameter to define the presence of inflammation has therefore been intensive for many decades. Recently, interest has focused on the acute phase proteins (APPs) as indicators of inflammation. APPs can be separated into positive APPs which increases in the presence of inflammatory triggers, and negative APPs which decreases in acute phase state (Pepys *et al*.,1983). Positive APPs can be further divided into major APPs which increases either 10, 100 or even 1000 times fold in the presence of inflammatory triggers, and minor APPs which increases very little (Pepys *et al*., 1983). Among positive APPs, serum amyloid A (SAA) is one and only identified major APP in horses (Mozes *et al*., 1989).

SAA is synthesised in the liver and its production decreases immediately after the synthesis stops or after recovery (Uhlar *et al*., 1999). It has low basal values with a narrow reference range (Kent, 1992). It quickly increases 10 to 1000 times its normal concentration in response to infection or inflammatory conditions, and decreases quickly in response to treatment. However it remains high if there is no recovery (Kent, 1992).

Since endurance horses continuously undergoing vigorous training in hot and humid Malaysian weather, the aim of this study is the determine the effect of resting interval on

concentration of serum amyloid A, and to arrive with the reference range value of this protein in clinically normal endurance horses in Malaysia.

2.0 LITERATURE REVIEW

2.1 Inflammation

Inflammatory response does not always followed by the classical clinical signs such as *dolor, rubor*, *calor, tumor* and *funtio laesea* (Jacobsen *et al*., 2007). Thus, recognising inflammation in a horse that do not display obvious clinical signs yet remain ill is a challenge. Blood biochemistry or haematological testing may be needed in order to identify inflammatory disease (Jacobsen *et al*., 2007). Presence of acute phase proteins (APP) in the blood becomes an indicator for the occurrence of inflammation. Acute phase proteins comprises of a large and very heterogeneous group of proteins, among which serum amyloid A (SAA) seems to be a particularly sensitive marker of inflammation (Mozes *et al*., 1989).

2.2 Acute phase response (APR)

The acute phase response (APR) is the fast and non-specific response brought up by all sorts of inflammatory stimuli, such as infection, trauma, cancer, and ischemia (Kushner *et al.*, 1993). The APR is started when affected cells and tissues release 'alarm molecules' such as reactive oxygen species, arachidonic acid, metabolites and modified host proteins recognised as foreign, and thereby activate monocytes and macrophages to produce a large number of inflammatory mediators, among which the cytokines play very important roles (Koj, 1996). The local, paracrine effects and the distant, endocrine effects of cytokines propagate the APR cascade by stimulating several other cell types.

During the APR, the metabolism is directed at removal of the inflammatory stimulus, promotion of healing and repair process, and restoration of homeostasis (Jacobsen *et al*., 2007). A central pathophysiological step of the APR is hepatic synthesis and hence increased plasma concentration of acute phase proteins (APPs) (Jacobsen *et al*., 2007).

2.3 Acute phase proteins (APPs)

Acute phase proteins are proteins whose plasma concentrations change during the APR towards infectious and non-infectious disease (Gabay *et al*., 1993). The group of positive APPs, whose plasma concentrations increase during APR, are divided according to their pattern of response to stimulation (Jacobsen *et al*., 2006). The major APPs are those that have very low or undetectable levels in plasma of healthy individuals, and whose plasma concentration increase more than 10 and often 100 or 1000 times during acute phase states (Jacobsen *et al*., 2006). Serum amyloid A is the only major APP identified in the horse to date (Jacobsen *et al*., 2006). The minor and moderate APPs are those that are already present in plasma of healthy individuals, and whose concentration increase only 1- 10 times during the APR (Jacobsen *et al*., 2006).

Some of APP plasma concentrations decrease during the APR and it is called negative APP. In horses and many other species, albumin is a negative APP (Allen and Kold, 1988). During the APR the demand for amino acids for synthesis of positive APPs is markedly increased, which necessitates reprioritisation of hepatic protein synthesis (Aldred and Scheiber, 1993).

The APPs have different kinetic profiles; some increase within a few hours of infection or tissue injury and reach peak values within 1 or 2 days, whereas others have a slower increase and remain elevated for longer (Jacobsen *et al*., 2007). In horses, SAA is an example of the APP that reached peak values within 1 or 2 days (Nunokawa *et al*., 1993).

2.4 Equine SAA protein

Three isoforms of SAA have been detected in acute phase serum from horses (Hultén *et al*., 1997). SAA is degraded in the liver and its plasma half-life is very short, and plasma levels of SAA thus decrease soon after synthesis ceases (Uhlar *et al*., 1999). Several functions of serum amyloid A have been described, namely to enhance and inhibit several leucocyte functions, to influence synthesis of inflammatory mediators, to affect lipid

transportation to inflamed tissue, and to induce enzymes involved degradation of extracellular matrix (Jacobsen *et al*., 2007).

SAA has low basal values with a narrow reference range which remains unchanged with age, sex, or genetic make-up of horses (Kent, 1992). It quickly increases to very high values (more than 100X) in response to infection or inflammatory conditions and decreases quickly in response to treatment but do not decrease if there is no recovery (Kent, 1992).

Plasma SAA concentrations of healthy horses have been reported to range from less than 0.5-20 mg/l (Jacobsen *et al*., 2006). Neonatal which is less than one week old and horses older than 8 years old showed slightly higher SAA levels compared to other age group animals (Nunokawa *et al*., 1993). Serum amyloid A levels do not differ between genders in the horse, nor do they seem to be significantly influenced by pregnancy (Nunokawa *et al*., 1993). After parturition, SAA levels increase transiently, probably due to tissue damage inflicted on the birth canal during expulsion of the foetus. There were no changes in SAA concentrations after race training in experienced horses. In horses that were beginning their endurance training, exercise produced an increase in SAA level as compared with rest level (Cywinska *et al*., 2013).

3.0 MATERIALS AND METHODS

3.1 Test subjects

A total of 40 clinically healthy endurance horses from three stables namely Selangor Turf Club, Terengganu State Endurance Stable and Skuadron Istiadat Berkuda, were utilised in this study. All the horses were either Arabian or Thoroughbred breeds.

Twenty geldings, 16 mares and 4 stallions, aged between 6 to 22 years old, had previously participated in endurances competition at least 6 months but not more than 43 months prior to this study. All individuals were still undergoing training and must not be lame upon blood sampling. Horses have to be clinically normal on physical examination and on the basis of previous history.

3.2 Blood samples

Blood samples were taken from the jugular vein, when horses were in their stalls. Ambient temperature and resting environment were noted during the time of sampling. Jugular vein were occluded and swabbed with alcohol to sterilise the venipuncture region. Samples were collected in two 3ml plain tubes.

Sampled blood was allowed to clot for 20 minutes and was centrifuged at 3400 × g for 10 minutes. Serum was separated and transferred into 1ml Eppendorf™ tubes. The serum was then stored at -20 ℃ before SAA was assayed using a two-site enzyme linked immunoassay (EIA) test (Horse SAA ELISA, Immunology Consultants Laboratory, Inc).

3.3 Serum assay

The assay for quantification of SAA in samples required each test sample be diluted at 1/200 before use. To prepare a 1/200 dilution of sample, 2 µL of sample was transferred to 398 µL of 1 X diluent. The mixture was mixed thoroughly.

All reagents were brought into room temperature before use. 100 µL of standard 0 (0.0 ng/ml), standard 1 (2.25 ng/ml), standard 2 (4.5 ng/ml), standard 3 (9 ng/ml), standard 4 (18 ng/ml), standard 5 (36 ng/ml) and standard 6 (72 ng/ml) were pipetted in duplicate in the well. 100 µl of diluted samples were pipetted into pre designated wells. The micro titre plate was incubated while shaking on an orbital shaker at room temperature for 60 minutes. The plate was covered and levelled during incubation.

The content of the wells were aspirated after incubation. The micro titre plate was washed using washer with wash solution. 100µL of diluted detection antibody was pipetted into each well. The micro titre plate was incubated while shaking on an orbital shaker at room temperature for 20 minutes in a dark room. The plate was covered and level during incubation.

The content was aspirated and washed using washer with wash solution followed by blotting of the wells. 100µL of diluted HRP-strepavidin was pipetted into each well. The micro titre plate was incubated while shaking on an orbital shaker at room temperature for 20 minutes in a dark room. The plate was covered and level during incubation.

The content was aspirated and washed using washer with wash solution followed by blotting of the wells. 100µL of TMB substrate solution was pipetted into each well. The micro titre plate was incubated while shaking on an orbital shaker at room temperature for 10 minutes in a dark room.

After 10 minutes, 100 µL of stop solution was added to each well. The absorbance (450nm) of the contents of each well was determined. The plate was calibrated according to manufacturer's specifications.

3.4 Statistical analysis

Mean concentrations of SAA, age, sex and breed of the endurance horses were tabulated into Microsoft® Excel 2010. Statistical analysis, means and standard errors of mean were computed using SPSS® 20 for Windows® Microsoft. The results are expressed

as the mean± standard error of the mean (SEM). The difference in SAA concentrations were evaluated between age groups, sex and breeds.

4.0 RESULT

Of the 40 horses sampled in this study, 20 individuals (50%) were geldings, 16 individuals (40%) were mares and 4 (10%) were stallions. Prior to the start of this study, 16 individuals (40%) had raced at least 6 months ago, 14 individuals (35%) had been involved an endurance race between 6 and 12 months ago, 4 individuals (10%) that had raced in an endurance competition between 12 and 24 months ago, and 6 individuals (15%) had not raced more than 24 months ago..

There were 14 horses (35%) that were aged between 6 and 11 years old, 20 horses (50%) that were between 12 and 18 years old, and 6 horses (15%) that were more than 19 years old. Thirty six of the test subjects were Arabian horses while the remaining 4 were Thoroughbred horses.

4.1 SAA concentration based on rest duration

Horses which had last participated in any endurance race between 12 and 24 months prior to this study expressed the highest mean serum amyloid A (SAA) concentration of 7.22 ± 5.22 mg/l. (Figure 4.1). Individuals that had last competed at least 6 months before had a mean SAA of 6.87 ± 1.74 mg/l, while horses which last raced between 6 and 12 months ago, had a mean SAA concentration of 3.71 ± 0.44 mg/l (Figure 4.1). The lowest mean SAA concentration was recorded among horses that had been rested more than 24 months prior to the start of this study, at 2.62 ± 0.30 mg/l (Figure 4.1).

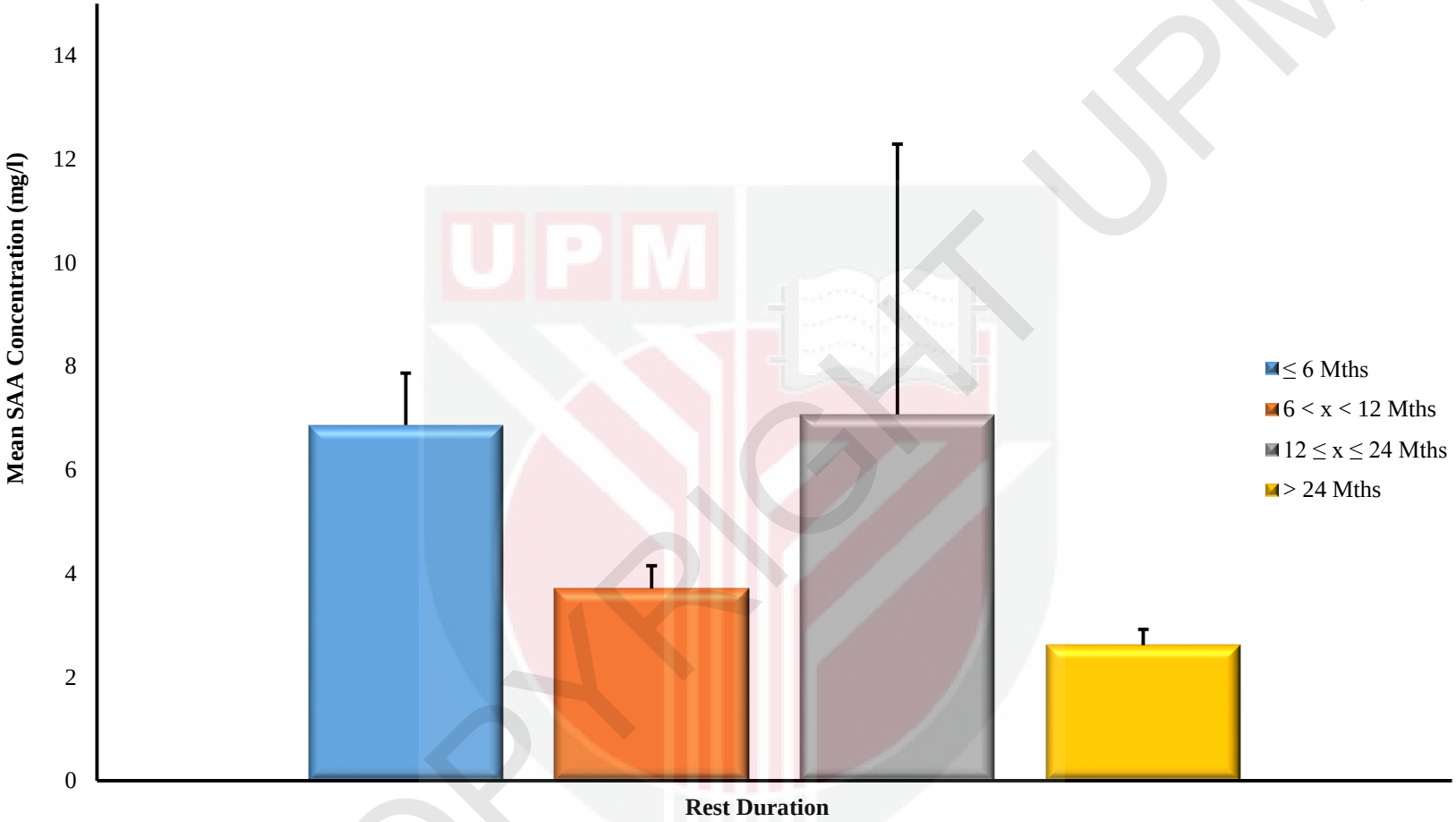

Figure 4.1 Comparative mean SAA concentration (mg/l) of clinically normal endurance horses (n = 40) at four periods from three stables.

4.2 SAA concentration based on age

The highest mean SAA concentration of 8.38 ± 2.25 mg/l was recorded in the group aged between 6 and 11 years old (Figure 4.2). Mean SAA concentration in horses more than 19 years old was 3.80 ± 0.55 mg/l (Figure 4.2). However the lowest mean SAA concentration was measured in horses between 12 and 18 years of age, at 3.28 ± 0.42 mg/l (Figure 4.2). There was a significant difference in the mean SAA concentration between the horses that were between age group 6 and 11 years old and those that were between 12 and 18 years old (Figure 4.2).

4.2.1 SAA concentration based on age according to rest interval

When mean SAA concentration (mg/l) was compared with age according to resting interval, the highest mean SAA concentration was noticed in younger horses that were between 6 years and 11 years of age. These horses had competed in endurance race between 12 and 24 months before sampling commenced (Figure 4.3) and thus expressed a mean SAA concentration of 22.53 mg/l. The next highest value was noticed in horses participated in endurance races, less than or equal to 6 months with mean SAA concentration of 9.22 mg/l followed by in horses participated in endurance race between 6 and 12 months with mean SAA concentration of 4.55 mg/l. The lowest mean SAA concentration noticed in horses participated in endurance race more than 24 months with value of 2.88 mg/l (Figure 4.3).

4.3 SAA concentration based on gender

When mean SAA concentration (mg/l) was compared against gender, mares were recorded to have the highest mean SAA concentration at 7.33 ± 1.96 mg/l (Figure 4.4). Whereas, stallions and geldings both expressed similar mean SAA concentrations of 3.78 ± 0.79 mg/l and 3.67 ± 0.66 mg/l, respectively (Figure 4.4).

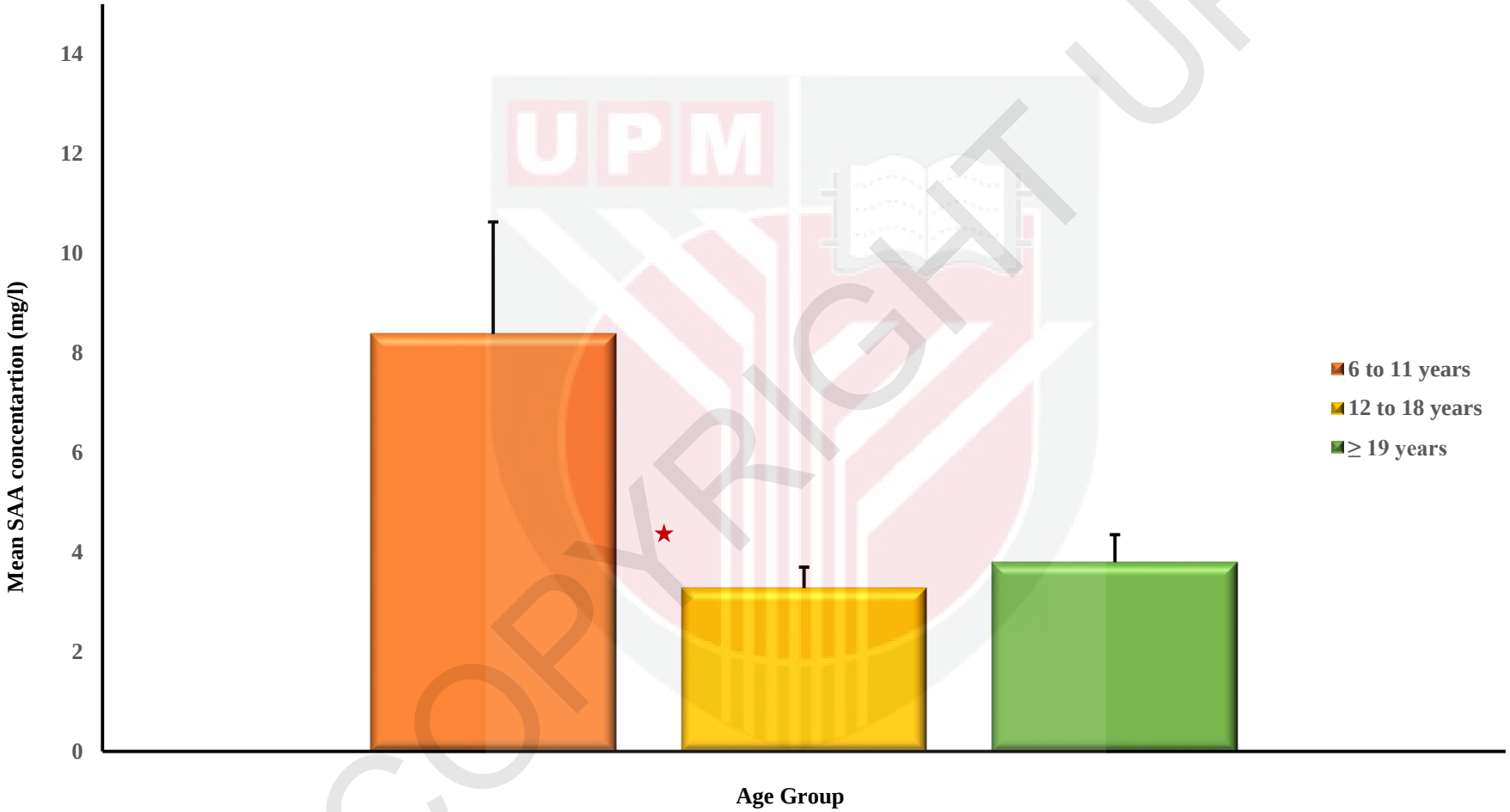

Figure 4.2 Comparative mean SAA concentration (mg/l) of clinically normal endurance horses (n = 40) at three age group from three stables. The asterisk indicates significant mean difference.

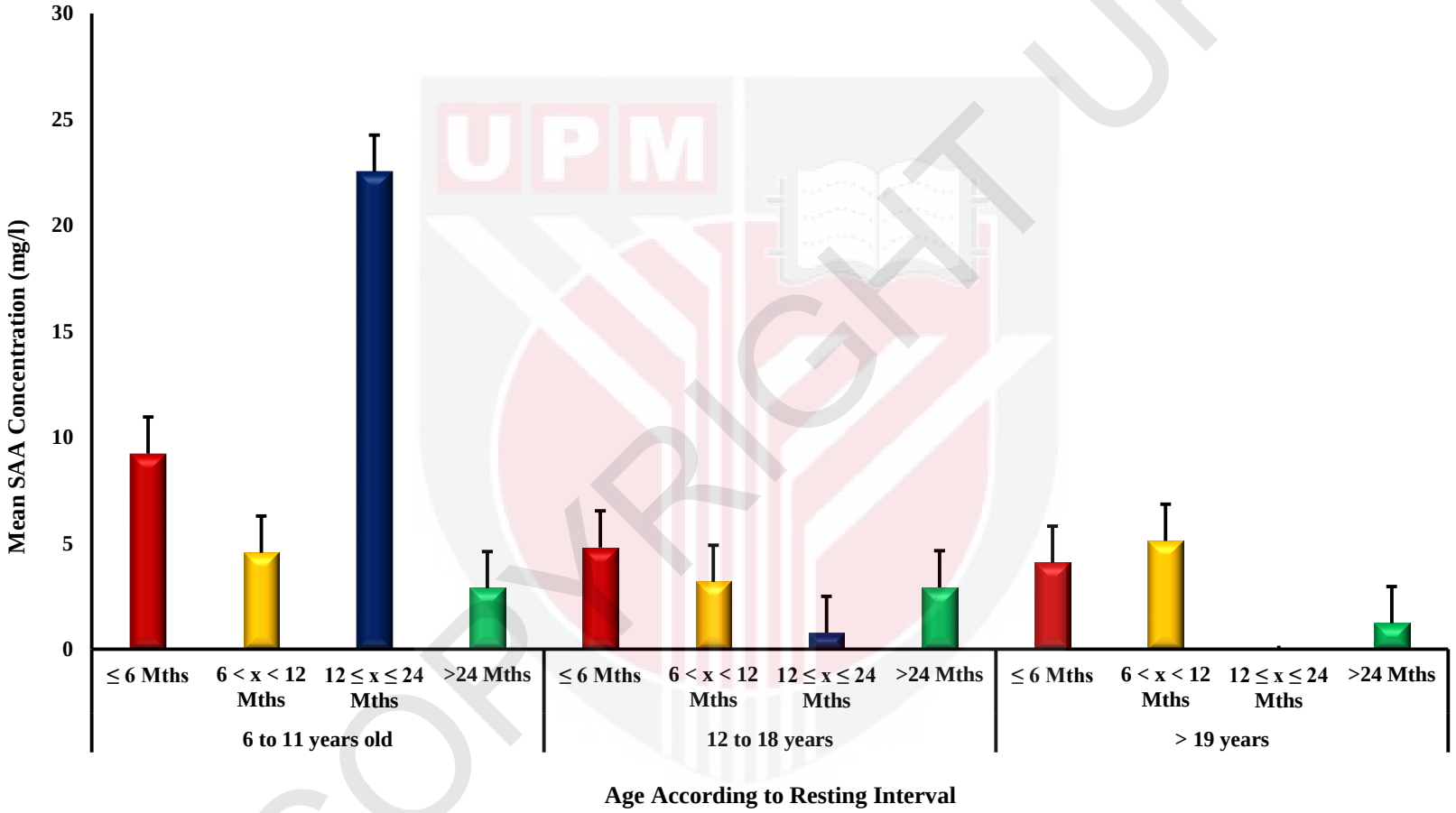

Figure 4.3 Comparative mean SAA concentration (mg/l) of clinically normal endurance horses (n = 40) at four age groups according to their rest interval, from three stables.

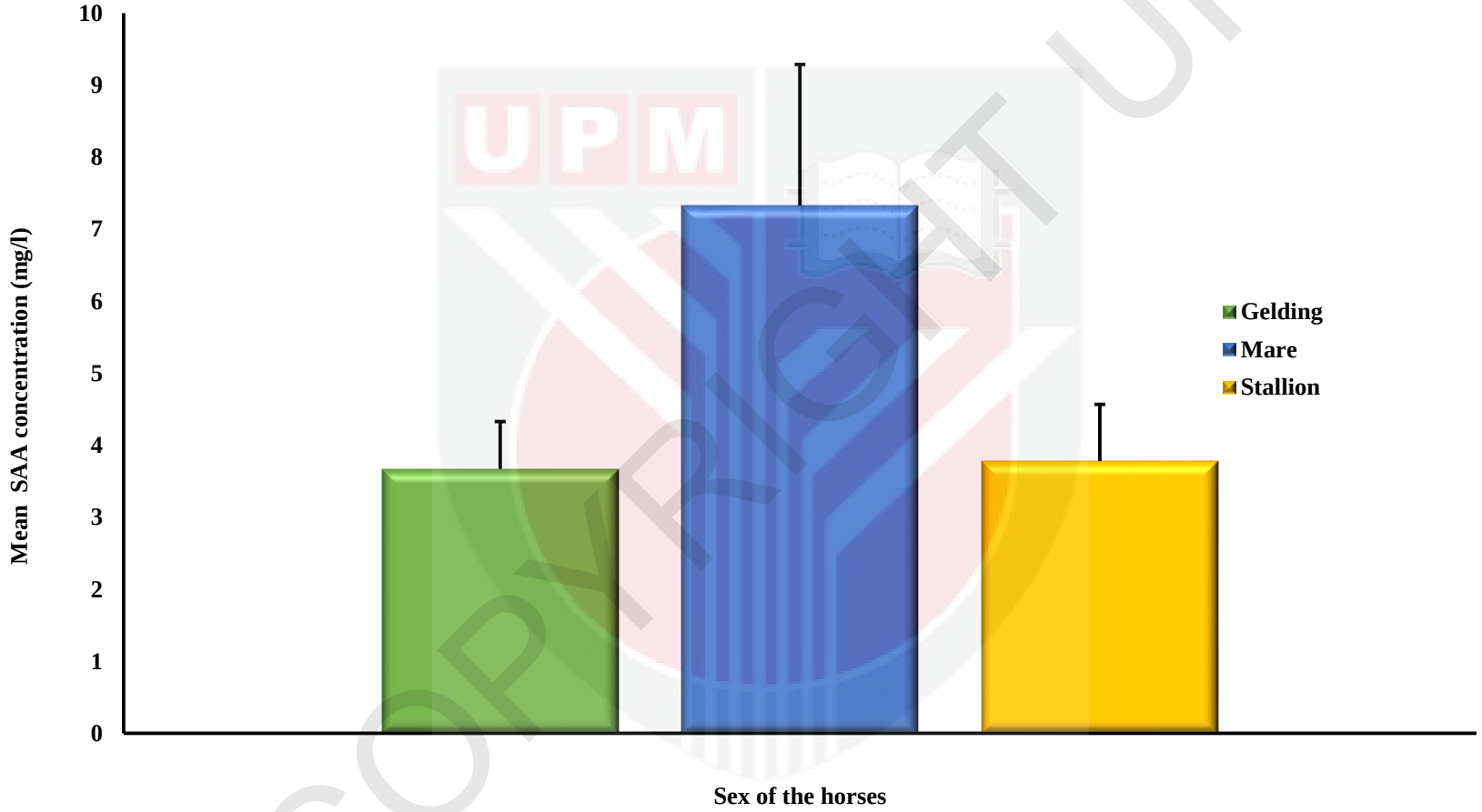

Figure 4.4 Comparative mean SAA concentration (mg/l) between clinically normal endurance geldings (n = 20), mares (n = 16) and stallions (n = 4).

4.3.1 SAA concentration based on gender according to rest interval

Mares that had last raced between 12 and 24 months prior to this study had the highest SAA concentration which is 22.53 mg/l, followed by mares that participated in endurance race less than or equal to 6 months before with value of 8.3 mg/l, and followed by mares participated between 6 months and 12 months with value of 4.88 mg/l and the lowest mean SAA concentration was noticed in mares rested more than 24 months with SAA value of 2.59 mg/l (Figure 4.5).

Whereas in geldings, the mean SAA concentration were high among the groups that participated in endurance less than or equal to 6 months before with SAA value of 5.04 mg/l and it decreased as the geldings rested longer (Figure 4.5).

4.4 SAA concentration based on breed of horses

There was a significant different of the mean SAA concentration between Thoroughbred and Arabian horses (Figure 4.6). Thoroughbred horses have almost twice the mean SAA concentration compared to Arabian horses (Figure 4.6). The mean concentration for Thoroughbred and Arabian horses were 9.25 ± 3.95 mg/l and 4.57 ± 0.82 mg/l, respectively (Figure 4.6).

4.4.1 SAA concentration based on breed according to rest interval

Among the Thoroughbred horses, the horses participated from 12 to 24 months before have high mean SAA concentration with value 22.53mg/l, followed by horses participated less than or equal to 6 months before with value of 8.14 mg/l and horses participated between 6 and 12 months before with value of4.22 mg/l (Figure 4.7).

The mean SAA concentration for Arabian horses was the highest for the horses participated less than or equal to 6 months with value of 6.69 mg/l, followed by horses participated between 6 and 12 months with value of 3.67 mg/l. The next high SAA

concentration was noticed in the group of horses participated more than 24 months with value of 2.62 mg/l. The lowest mean SAA was observed in the group of horses participated from 12 to 24 months with value 1.92 mg/l (figure 4.7).

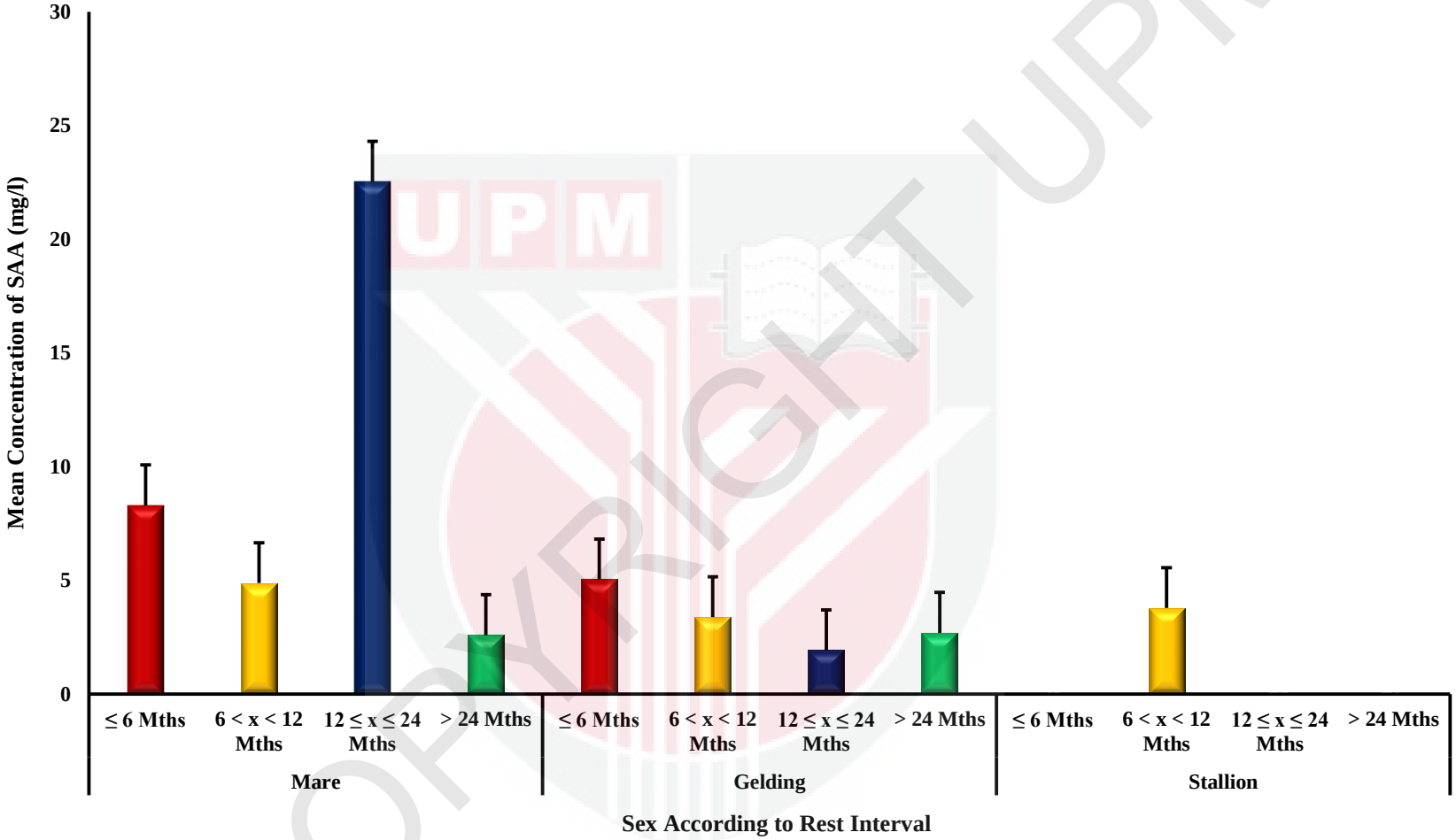

Figure 4.5 Comparative mean SAA concentration (mg/l) between clinically normal endurance geldings (n = 20), mares (n = 16) and stallions (n = 4) according to rest interval.

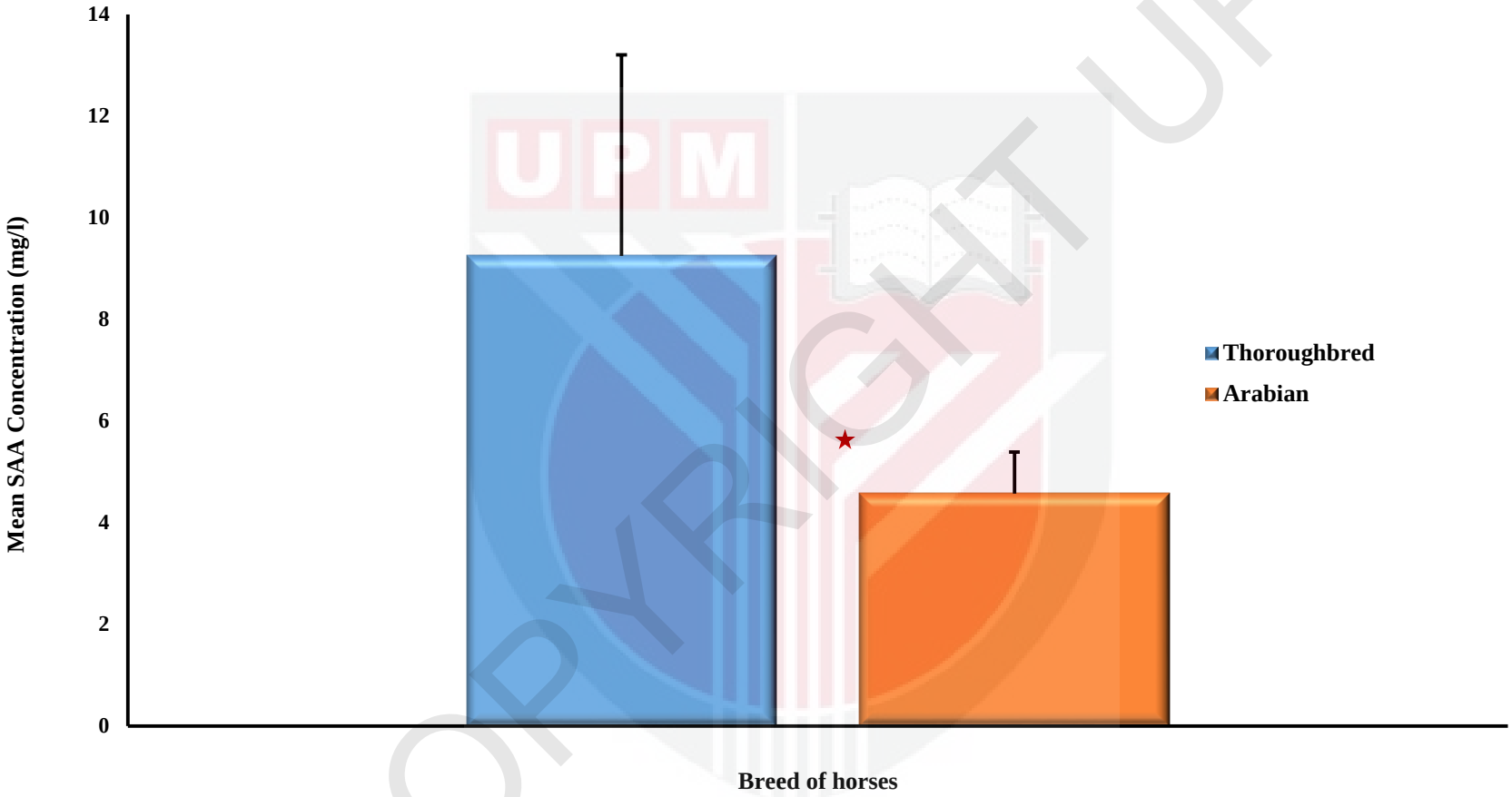

Figure 4.6 Comparative mean SAA concentration (mg/l) between Thoroughbred (n = 4) and Arabian (n = 36) endurance horses. The asterisk indicates significant mean difference.

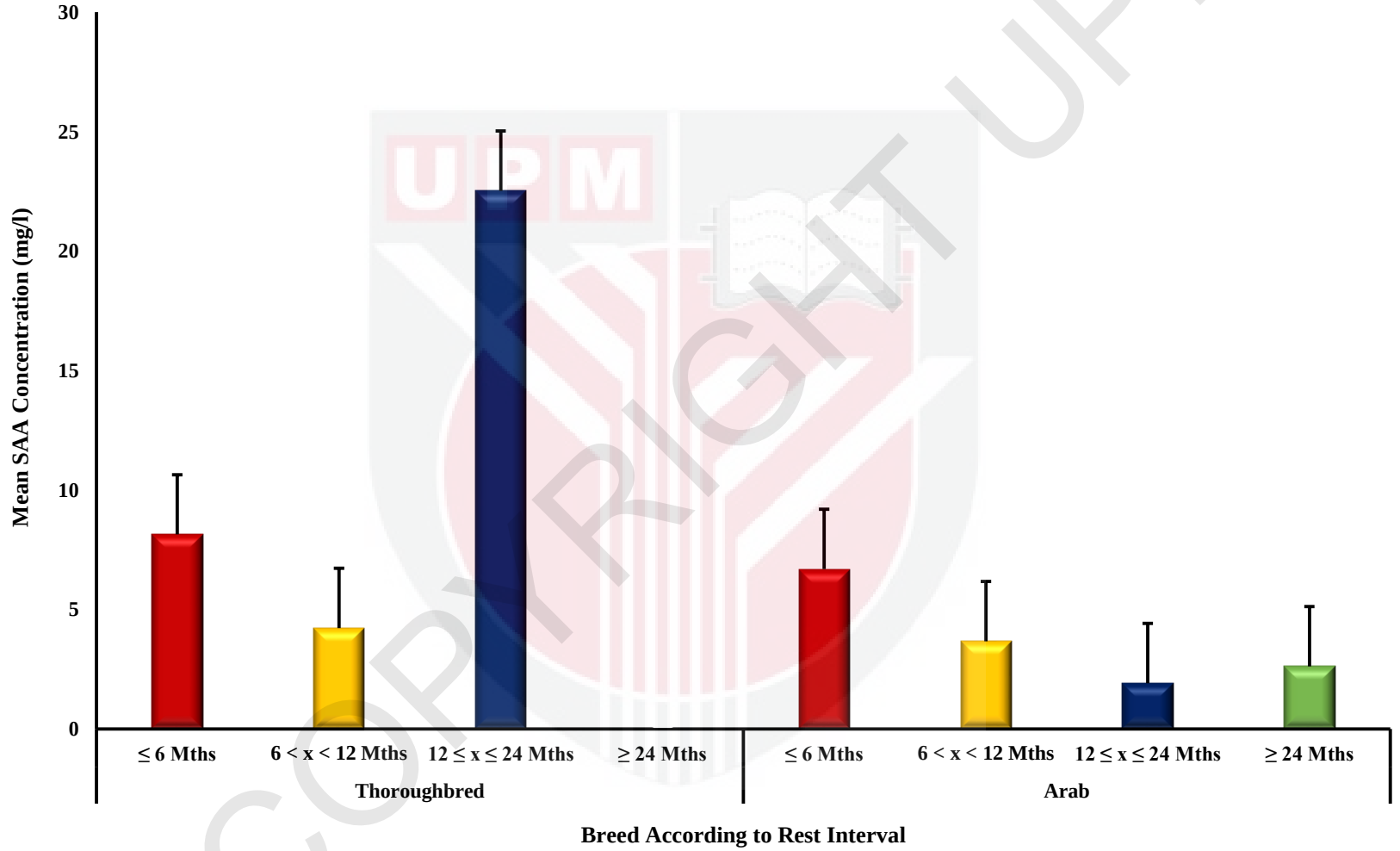

Figure 4.7 Comparative mean SAA concentration (mg/l) between Thoroughbred (n = 4) and Arabian (n = 36) endurance horse according to rest interval.

5.0 DISCUSSION

The present study was undertaken to establish the level of SAA in clinically normal endurance horses. Several factors had been taken into considerations, namely (i) the effect of rest interval on mean SAA concentration, (ii) the effect of age according to rest interval on mean SAA concentration, (iii) the effect of gender according to rest interval on mean SAA concentration, (iv) the effect of breed according to rest interval on mean SAA concentration.

The mean SAA concentration for horses participated in endurance from 12 months to 24 months were high could be due to occurrence of injuries results from reduction in fitness. But the result obtained here is a bit controversial since just one horse shows very high SAA concentration which 22.5mg/l whereas other horses were ranging from 0mg/l to 4mg/l. Without this one horse with high SAA concentration, the mean SAA concentration will be decreasing in the group of horse participated less than or equal to 6 months before to group of horse participated more than 24 months before. The horses participated in endurance less than or equal to 6 months have high SAA concentration with the value of 6.87 mg/l could be related to the ongoing recovery process from the inflammatory reaction occurred during the endurance races that they participated previously (Cywińska *et al*., 2012). Inflammatory reaction could also be due to orthopaedic injuries. Examples of orthopaedic injuries can be caused by endurance races could be tear in forelimb suspensory ligament and superficial digital flexor tendons which could lead to prolonged recovery (Gomide *et al*., 2006). Metabolic condition cannot be included as in 6 months periods, the metabolic condition will be resolved (Foreman, 1998). The lowest mean SAA concentration was noticed in horses participated more than 24 months before with value 2.62 mg/l because most probably these horses have been retired from endurance and in this 24 months period, the horses will recover well from injuries or from any inflammatory triggers. Standard error mean is a measure of the statistical accuracy of an estimate, equal to the standard deviation

of the theoretical distribution of a large population of such estimates. So, in real population the SAA concentration will reach until the stated SEM value.

Younger horses aged between 6 and 11 years old exhibited higher mean SAA concentration. This may be because younger horses needed more adaption towards the endurance race and training which during this processes could increase the SAA concentration in response to inflammatory reaction (Giori *et al*., 2011). The SAA concentration for middle aged horses which is 12 years to 18 years old is the lowest among the groups. This result is in agreement with author (Giori *et al*., 2011), where horses at this age already adapted to the endurance race and they are fit for all the training they underwent. However, the older horses exhibit slightly higher SAA level from the middle aged horses, could due to decrease of elasticity of the musculature with age (Kenyon *et al*., 2007).

On the other hand, when the effect of gender on SAA concentration is compared, the geldings and stallions showed very low mean SAA concentration compared to mares because there are no stressful hormonal changes was occurring in stallions and geldings (Hultén *et al*., 1997). The mean SAA concentration is very high in mare could be explained due to ongoing oestrus cycle in mares, where such cycle does not occur in stallion and geldings. During proestrus and metestrus, mares tend to show very aggressive and stressful behaviour which could cause increase in SAA concentration (Hultén *et al*., 1997).

Mean SAA concentration on Thoroughbred horses was high compared to Arabian horses due to composition of mainly fast twitch muscle fibres in Thoroughbred horses (Thiruvenkadan *et al*., 2009). Fast twitch muscle fibres are low oxidative, meaning they are highly anaerobic. This fibre are used to give the horse speed and has no ability to reduce the accumulation of lactate. Accumulation of lactate will lead to fast exhaustion. Exertion beyond the exhaustion point will lead to increase in chances of injuries and causes increase in SAA level. Arabian horses on the other hand have slow twitch muscle fibres which is highly oxidative, meaning they use aerobic metabolism to produce energy-generating ATP. These fibres are used for endurance and are said to be “fatigue-resistant” because they are

capable of reducing the toxic end products of metabolism, such as lactate. Since endurance rides are long term, submaximal intensity aerobic events; therefore, more slow twitch fibers are required. This attributes of Arabian horses is beneficial to this breed as less exertion were exerted during endurance rides and reduce the inflammatory response which could reduce the SAA level (Adamu *et al*., 2014).

Based on the results, the suggestive reference range for endurance race horses in Malaysian climate could be from 2.01mg/l to 8.01 mg/l where 77.5% of the horses were within this range. Most of the endurance race horses will be resting less than 6 months, thus the reference range taken from the group of horses participated in endurance races are less than or equal to 6 months. Within this group itself, the percentage of horses having SAA concentration from 2.01mg/l to 8.01mg/l are 87.5%. Another 12.5% of animals having mean SAA concentration of more than 14 mg/l which could be ongoing inflammatory process.

6.0 CONCLUSION

Based on the study conducted it is suggestive that the reference range for endurance race horses in Malaysia would be ranging from 2.09mg/l to 8.01mg/l. This range of SAA concentration only applicable in Malaysia where the terrain and weather conditions are making the endurance race much tougher for the horses. More than the suggested SAA concentration indicates presence of inflammatory response and can be recommended for longer resting period and veterinary observation and attention

Middle aged horses, which is from 12 years to 18 years in this research shows very low inflammatory process compared to younger and older horses. In this context, horses from the age 12 years and onward with continuous training from the younger age could perform well in endurance horses.

Arabian geldings and stallions showed low inflammatory response, thus usage of Arabian geldings or stallions would be beneficial for the rider to guide the horse and to endure the race smoothly.

7.0 RECOMMENDATIONS

The recommended SAA reference range for endurance horses in Malaysia may be between 2.09 and 8.09 mg/l. As the endurance terrain and weather are tougher in Malaysia, it is expected that the SAA concentration in endurance horses in Malaysia could be higher than other places.

Best recommended breed for endurance race would be Arabian horses compared to Thoroughbred horses as the Arabian horses are bred for exhaustion resistant and composed of muscle fibres type 2 which is completely aerobic. Aerobic energy production does not involve accumulation of lactate which gives extra time or prolong the time taken for the horse to reach exhaustion period.

Stallion and gelding are recommended animals as they will not undergo hormonal changes which will lead to prolong stress unlike in mares.

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